ImmunoTools *special* Award 2015

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Dissection of the immune response induced by a recombinant vaccine against *Fasciola hepatica* in rodents.

Background

Fasciolosis is an important freshwater snail-borne helminthiasis caused by the trematode parasites *Fasciola hepatica and F. gigantica* that produces a chronic liver infection of cattle and sheep. Both species inflict substantial productive loses on affected animals estimated globally at 3.2 billion USD mainly due to reductions in meat, wool and milk output of infected animals, with additional costs resulting from liver condemnation and the use of flukicide drugs. While *F. hepatica* has been recorded in all continents, *F. gigantica* is restricted to Africa and Asia. In addition, human fasciolosis caused by *F. hepatica* has been recently recognized as an emerging/re-emerging zoonotic disease in many countries with an estimated prevalence of up to 17 million people infected and 180 million at risk of infection worldwide.

Currently, fasciolosis control is primarily dependent on repeated treatment with anthelmintic drugs and pasture management. Although effective drugs such as triclabendazole are available, they provide only temporary disease control of the disease, as cattle and sheep are easily reinfected. Moreover, triclabendazole-resistant strains have appeared, quickly after the introduction of the drug, in parts of Europe and Australia. Furthermore, there is growing consumer concern over the routine use of drugs in food-producing animals. Accordingly, these problems highlight the need for effective vaccines to control fasciolosis that will result in an improved production economics and animal welfare.

Although protection experiments against liver flukes using homologous infections in ruminants started more than 50 years ago, defined immunogens were characterized and used in vaccine trials less than 2 decades ago. Since then, most vaccination experiments using defined components, either purified native or recombinant proteins, have been carried out using proteases, hemoglobin, glutathione S-transferase or fatty acid binding proteins as immunogens. In this context, more than a decade ago, leucine aminopeptidase (*Fh*LAP), a gut-associated exopeptidase isolated in our lab from a detergent soluble-extract of adult worms was successfully used as a vaccine in its native form against *F. hepatica* in Corriedale

sheep. Use of Freund´s adjuvant- *Fh*LAP alone or in combination with the adult stage-specific secreted cathepsin L proteases *Fh*CatL1 and *Fh*CatL2 induced high levels of protection. Vaccinated animals in the *Fh*LAP group showed an 89% reduction in worm burden compared to the control group, with 4 of 7 sheep harboring no flukes in their livers, one of the highest levels of protection yet obtained in ruminants.

More recently, a fully functional recombinant *Fh*LAP expressed in *Escherichia coli* as a thioredoxin fusion protein was molecularly and biochemically characterized, and was found to be identical to the previously isolated immunogen. When subcutaneously inoculated mixed with Freund's adjuvant, r*Fh*LAP induced a strong protective immune response in rabbits that were orally challenged with *F. hepatica* metacercariae.

More recently r*Fh*LAP mixed with different adjuvants (Freund´s, Alum, Adyuvac 50, DEAE-D and Ribi) induced significant levels of protection in sheep against metacercarial challenge. Particularly relevant were the protection levels conferred by vaccination with this recombinant exopeptidase in combination with Freund´s (84%), Alum (87%) and Adyuvac 50 (74%).

A number of vaccine trials have shown a relationship between the development of significant protection levels against *F. hepatica* and a specific Th1 response in contrast to the non-protective Th2 response observed during experimental infection. However we found a consistent mixed IgG1/IgG2 response in immunized sheep. In addition, the anti-rFhLAP humoral response elicited in control animals, albeit very low, showed a similar pattern.

Experimental plan

The aim of the present study is further dissect the humoral and cellular response induced by the vaccination of mice with a functional recombinant FhLAP cloned in pET28a vector and expressed in *E. coli*. To this purpose we will conduct an immunization experiment in BalbC mice using Adyuvac 50 and Alum as adjuvants. Eight weeks-old mice allocated into 5 groups of 10 each will be intraperitoneally inoculated with 25 ug of the hexameric functional rFhLAP mixed with Alum (G1) or Adyuvac 50 (G2). Controls groups will be similarly inoculated with PBS in both adjuvants (G3, G4) or PBS alone (G5) at weeks 0, 4 and 8. Animals will be challenged with 10 *F. hepatica* metacercariae on week 10 and necropsied at week 14. Blood and lymph node tissue samples will be used to evaluate mechanisms of immune responses induced by vaccination compared with the non-vaccinated ones. The immune response will be investigated evaluating humoral response (serum specific IgG1, IgG2a, IgG2b, IgG2c, IgE) by ELISA, and tissue T cells, B cells, NK cells, macrophages and neutrophils will be assessed by flow cytometry (Immunotools). In addition purified peritoneal macrophages obtained at different time points will be incubated with r*Fh*LAP or LPS and the level of IL12-p40 and NO will be measured in culture supernants.

ImmunoTools *special* AWARD for **Carlos Carmona** includes 25 reagents

FITC - conjugated anti-mouse CD3e, CD4, CD8a, CD11b, CD19, CD25, Gr-1, NK-cells, isotype control IgG2b,

PE - conjugated anti-mouse CD3e, CD4, CD19, CD25, Gr-1, NK-cells, isotype control IgG2b,

APC - conjugated anti-mouse CD3e, CD4, CD8a, CD11a, CD19, Gr-1, NK-cells,

recombinant mouse cytokines: rm IFNgamma, rm IL-4 DETAILS more AWARDS